Evolutionary Systematics. 5 2021, 25–38 | DOI 10.3897/evolsyst.5.62929

PENSOFT

EVOLUTIONARY SYSTEMATICS

Dwarfs of the fortress: A new cryptic species of dwarf gecko of the genus *Cnemaspis* Strauch, 1887 (Squamata, Gekkonidae) from Rajgad fort in the northern Western Ghats of Maharashtra, India

Amit Sayyed[1], Vivek Philip Cyriac[2,3], Anish Pardeshi[4], Shauri Sulakhe[5]

1 *Wildlife Protection and Research Society (WLPRS), Maharashtra, India*
2 *The Liana Trust, Sy. No. 1418/1419, Rathnapuri, Hunsur – 1571189, Karnataka, India*
3 *Centre for Ecological Sciences, Indian Institute of Science, Bangalore – 560012, Karnataka, India*
4 *Sadashiv Peth, Vishnu Apartment, Pune, Maharashtra, India*
5 *Institute of Natural History Education and Research (INHER), C-26/9, Ketan Heights, Kothrud, Pune, Maharashtra – 411038, India*

http://zoobank.org/30DCC409-5E6F-4460-B64F-33C9E9F74EB8

Corresponding author: Shauri Sulakhe (shaurisulakhe@gmail.com)

Academic editor: Alexander Haas ♦ **Received** 8 January 2021 ♦ **Accepted** 2 March 2021 ♦ **Published** 19 March 2021

Abstract

A new species of the genus *Cnemaspis* Strauch, 1887 is described from the Rajgad fort in the northern Western Ghats of Maharashtra, India. The new species, belonging to the *flaviventralis* clade, is one of the smallest known Indian *Cnemaspis* and can be distinguished from other congeners by its genetic distinctiveness and few key morphological characters. The new species can be diagnosed from all other Indian congeners by its small body size (SVL < 27 mm), the absence of conical and spine-like tubercles on flank; heterogeneous dorsal pholidosis; presence of only femoral pores and no precloacal pores in males; weakly keeled scales on the ventral surface of neck, pectoral, abdominal region and limbs; granular scales on the tail with whorls of slightly enlarged, strongly keeled tubercles; and the absence of enlarged median subcaudal scales. The new species is currently known to inhabit the man-made historical structures from a single locality in Rajgad, Maharashtra, where it is presumed to be uncommon.

Key Words

Cnemaspis rajgadensis sp. nov., taxonomy, dwarf gecko, cryptic species, reptiles, forts of Maharashtra

Introduction

Western Ghats of India is a known global biodiversity hotspot with high levels of endemism (Myers et al. 2000). The Western Ghats (WG) is a long but interrupted chain of mountains stretching from southern Gujarat to the southern part of Kerala and Tamil Nadu. This long mountain chain is interrupted by three main gaps, which divide the WG into the northern, central and southern WG. The Northern Western Ghats (NWG) starts from the Dang Range of Gujarat in the north and extends up to Goa in the south (Jog et al. 2002). Although, the northern WG is thought to be depauperate compared to the southern WG (Gunawardene et al. 2007), ongoing taxonomic research are continuously adding new species of reptiles and amphibians to the endemic diversity of this region (e.g. Sharma 1976; Padhye et al. 2013; Mirza et al. 2014; Deepak et al. 2016; Sayyed et al. 2016; Giri et al. 2019a, b; Khandekar et al. 2019).

The genus *Cnemaspis* Strauch, 1887 is one of the most diverse clades of geckos in India and recent studies have indicated that the diversity documented within this clade is far from complete (Khandekar 2019; Agarwal et al. 2020; Cyriac et al. 2020). The diversity of *Cnemaspis* is also exceptionally high in the NWG and recent studies have been describing several new species from the region (Sayyed et al. 2016, 2018; Khandekar et al. 2019; Sayyed and Sulakhe 2020). Currently 11

Copyright *Amit Sayyed et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

species of *Cnemaspis* are known from NWG of Maharashtra which fall into three separate clades – *wynadensis* clade, *goaensis* clade and *giri* clade. These include *C. amba* Khandekar, Thackrey & Agarwal, 2019, *C. kolhapurensis* Giri, Bauer & Gaikwad, 2009 and *C. ranganaensis* Sayyed & Sulakhe, 2020 in the Kolhapur region; *C. ajijae* Sayyed, Pyron & Dileepkumar, 2018, *C. koynaensis* Khandekar, Thackrey & Agarwal, 2019 and *C. girii* Mirza, Pal, Bhosale & Sanap, 2014 in the Satara region; *C. amboliensis* Sayyed, Pyron & Dileepkumar, 2018, *C. limayei* Sayyed, Pyron & Dileepkumar, 2018 and *C. flaviventralis* Sayyed, Pyron & Dahanukar, 2016 in the Sindhudurg region; *C. mahabali* Sayyed, Pyron & Dileepkumar, 2018 from Pune and *C. goaensis* Sharma, 1976 from southern Maharashtra and Goa. Except for *C. goaensis* and *C. mahabali*, all the species from NWG are known to have restricted ranges around their respective type localities.

The NWG in Maharashtrtra, popularly known as Sanhyadri, has been a region of strategic importance in several historical events due to its unique climatic conditions and geographic features. Many empires have occupied and ruled Maharashtra building more than 300 forts in NWG at vantage points on the lofty cliffs of the NWG, which are surrounded by dense forests (Pai 2005; Akkalkot 2009). Most of these forts are accessible only on foot, and are protected under the law as archeologically important sites making them and the surrounding areas naturally protected areas from extensive developmental activities. Thus, providing sanctuary to several endemic flora and fauna. The fortified structures, having several crevices, provide ideal habitats for several species of reptiles (Pal et al. 2013; Sayyed and Sulakhe 2020). During one of our surveys of the herpetofauna of these forts, we came across a distinct population of *Cnemaspis* from Rajgad fort, closely resembling *C. ajijae*. Using an integrated taxonomic approach, we here describe this population as a new species.

Materials and methods

Sampling

Specimens were collected during September 2020, in parts of Rajgad Fort, Pune District, Maharashtra, India. A single adult male and two gravid females were collected by hand, photographed in life, and euthanized using halothane. Tail clips were collected as tissue sample and stored in absolute ethanol for genetic analysis, after which specimens were fixed in 4% formaldehyde for 24 hours, washed in water, and transferred to 70% ethanol for long-term storage. Scalation and other morphological characters were recorded using a Lensel stereo microscope. The materials referred to in this study are deposited in the collection of the Bombay Natural History Society (BNHS) in Mumbai.

Molecular analysis

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from muscle tissue (tail clips) using a MACHEREY-NAGEL NucleoSpin DNA Insect kit following the manufacturer's protocols. A 550–600 base pair (bp) fragment of the mitochondrial 16S rRNA gene was amplified by standard 3-step polymerase chain reaction (PCR) using published primers (Palumbi et al. 1991). The fragment were amplified by 16SF (CGCCTGTTTATCAAAAACAT) and 16SR (CCGGTCTGAACTCAGATCACGT) primers with the following condition: 95 °C – 3 min,1 cycle, 95 °C – 30 sec, 50 °C – 30 sec, 72 °C – 1 min, 35 Cycles, 72 °C – 7 min. We then sequenced the forward and reverse DNA strands using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Sequences were manually checked and consensus sequence of 16S gene were generated from the forward and reverse sequence using BioEdit.

Sequence alignment

We added the newly generated sequences (see Table 1) to the 16S rRNA sequence matrix generated by Cyriac et al. (2020) for the Indian *Cnemaspis*. We excluded the sequence of *C. nilagirica* (MT217036) from the analysis, since it was found to be contaminated (Sayyed et al. 2020). Since out-group selection can influence phylogenetic reconstructions (Wheeler 1990; Gatesy et al. 2007), we used four species of *Lygodactylus* and three species of *Phelsuma* as out-groups following Cyriac et al. (2020). Accordingly, 60 in-group taxa, including the newly generated sequence of the new species, and seven out-group taxa were aligned using the MUSCLE algorithm (Edgar 2004) implemented in MEGAX (Kumar et al. 2018) using default parameters. The final alignment contained 72 sequences each of 604 bp length. We also calculated the pair-wise uncorrected p-distance for the 16S rRNA gene between sequences after complete removal of gap sites using MEGAX (Kumar et al. 2018).

Table 1. Voucher numbers and GenBank accession numbers for the sequence data generated in this study and used in the phylogenetic analysis.

Species	Voucher Number	GenBank accession number	Locality
Cnemaspis girii	ASPC6	MW682860	Morewadi, Satara
Cnemaspis girii	ASPC8	MW682861	Morewadi, Satara
Cnemaspis sp.	ASPC11	MW682862	Amba Ghat, Kolhapur
Cnemaspis sp.	ASPC12	MW682863	Amba Ghat, Kolhapur
Cnemaspis koynaensis	ASPC10	MW682864	Humbarali, Koyana, Satara
Cnemaspis koynaensis	ASPC9	MW682865	Humbarali, Koyana, Satara
Cnemaspis rajgadensis sp. nov.	BNHS 3100	MW682866	Rajgad fort, Pune
Cnemaspis rajgadensis sp. nov.	BNHS 3101	MW682867	Rajgad fort, Pune

Molecular phylogenetic analysis

We removed ambiguously aligned positions from the 16S rRNA sequence matrix using GBlocks v.0.91b (Castresana 2000). We then used PartitionFinder2 (Lanfear et al. 2016) on the 457 bp dataset to determine the best-fit substitution model. The dataset was not partitioned by codon position, given the non-protein coding nature of the marker. We performed a Maximum Likelihood analysis in IQ-tree (Nguyen et al. 2015) under the GTR + I + G model of sequence evolution, and branch support was tested using 1000 non-parametric standard bootstrap pseudo-replicates. Bayesian trees were generated using MrBayes v.3.2.6 (Ronquist et al. 2012) with two independent runs performed for five million generations sampling every 500 generations under the GTR + I + G substitution model. We ensured convergence of the two MCMC runs by ensuring that the standard deviation of split frequencies was less than 0.001 and by checking the trace plots in Tracer v. 1.7 (Rambaut et al. 2003). We also ensured that the ESS values for all the parameters were above 200. We discarded the first 25% of the trees and the best evolutionary hypothesis was inferred using the 50% majority consensus rule.

Morphological study

Morphological data were taken using a Yamayo digimatic calliper, a Mitutoyo 500, or a Tesacalip 64 to the nearest 0.1 mm. Morphological data recorded included the snout-vent length (SVL), distance from tip of snout to anterior margin of vent; trunk length (TRL), distance from axilla to groin; trunk width (TW), maximum width of the body; eye diameter (ED), horizontal diameter of the eye; eye-to-nares (EN), distance between anterior point of the eye to the posterior part of the nostril; snout length (ES), distance from anterior margin of the eye to the tip of the snout; eye-to-ear (ET), distance from posterior margin of the eye to the anterior margin of the ear opening; internarial distance (IN), least distance between the inner margins of the nostrils; ear opening diameter (EOD), horizontal distance from the anterior to posterior margin of the ear opening; head length (HL), distance from tip of snout to posterior edge of mandible; head width (HW), maximum width of the head; head depth (HD), maximum depth of the head; interorbital distance (IO), shortest distance between the superciliary scale rows; upper arm length (UAL), distance from axilla to elbow, lower arm length (FAL), distance from elbow to wrist; palm length (PAL), distance from wrist to the tip of the longest finger; finger length (FL), distance from the tip of the finger to the nearest fork; femur length (FEL), distance from groin to the knee; tibia length (TBL), distance from knee to heel toe length (TOL), distance from tip of 1st toe to the nearest fork; tail length (TL), distance between posterior margin of vent to the tip of the tail.

Meristic data recorded for all specimens included number of supralabials (SupL) and infralabials (InfL) on left (L) and right (R) sides; number of supraciliaries (SuS); number of interorbital scales (InO); number of scales between eye to tympanum (BeT), from posterior-most point of the orbit to anterior-most point of the tympanum; number of the postnasal (PoN),all scales posterior to the naris; number of postmentals (PoM);number of posterior postmentals (PoP), scales that are surrounded by the posterior-postmentals and between infralabials; number of supranasal (SuN), excluding the smaller scales between the larger supranasals; number of canthal scales (CaS), number of scales from posterior-most point of naris to anterior most point of the orbit; number of dorsal paravertebral scales (PvS), between pelvic and pectoral limb insertion points along a straight line immediately left of the vertebral column; number of mid-dorsal scales (MbS), from the centre of mid-dorsal row diagonally towards the ventral scales; number of mid-ventral scales (MvS), from the first scale posterior to the mental to last scale anterior to the vent; number of mid-body scales (BlS), across the ventral between the lowest rows of dorsal scales; femoral pores (FPores), the number of femoral pores; poreless scales (PS), number of poreless scales between left and right femoral pores; lamellae under digits of manus (MLam) and pes (PLam) on right (R) side, counted from first proximal enlarged scansor greater than twice width of the largest palm scale, to distal most lamella at tip of digits; lamellae under fourth digit of pes (LampIV). Morphometric data are given as % of SVL.

Morphometric analysis

We performed a multivariate analysis on morphometric variables collected from 19 specimens of the *flaviventralis* clade, which included eight specimens of *C. ajijae* and *C. flaviventralis* each and three specimens of the new species. We included only 15 of the 17 variables in this analysis because we were unable to obtain data for a few variables due to missing tails or digits (see Table 3). We carried out a Principal Component Analysis (PCA) in R v. 3.5.2 (R Core Team 2016) on the 15 variables after scaling all the variables in the data using the mean and standard deviation of the entire vector to reduce the dimensionality of the dataset and to identify variables contributing to the observed variation. We selected the first three principal components (PC), which together explained most of the variation and plotted the first and second, and the first and third PCs to visually examine the morphospace of the new species and the morphologically similar members of the *flaviventralis* clade.

Results

Phylogenetic relationships

The phylogenetic analyses recovered largely similar topologies for the Maximum Likelihood (ML) and Bayesian analyses. However, several clades were unresolved in both analyses. The new species from Rajgad fort was sister to *C. ajijae* with strong support (Bootstrap: 96; Posterior probability: 1.0) and together formed a clade with *C. flaviventralis* with moderate support (Bootstrap: 79; Posterior probability: 0.9). This clade formed a strong sister relationship with other members of the *girii* clade (*C. mahabali*, *C. amba*, *C. limayei*, *C. koynaensis* and *C. girii*), thus we ascribe the new species, *C. ajijae* and *C. flaviventralis* to a separate *flaviventralis* clade. Within the *girii* clade, (*C. girii* + *C. koynaensis*) and *C. limayei* formed a strongly supported clade which together was sister to *C. mahabali* with strong support. Interestingly, our samples collected from Amba village were nested within *C. mahabali*.

Uncorrected pairwise sequence divergence indicated a deep genetic divergence in the 16S rRNA gene between the *flaviventralis* clade and the rest of the *Cnemaspis* (p-distance > 7.7%). Intraspecific genetic divergences estimated for members of the *flaviventralis* clade were ca. 0.7% for *C. flaviventralis* and 0–1.45% for *C. ajijae* (Table 2). The new species was found to be deeply divergent from *C. flaviventralis* (p-distance = 7.0–7.4%) but showed shallow to moderate divergence with *C. ajijae* (p-distance = 1.9–2.7%) (Table 2).

Table 2. Comparison of intraspecific and interspecific uncorrected p-distance for three closely related species of the *Cnemaspis flaviventralis* clade (*C. flaviventralis*, *C. ajijae* and *C. rajgadensis* sp. nov.) for the 16S rRNA gene. 'N' indicates number of pairwise comparisons.

Pair-wise comparisons	N	Mean (%)	Range (%)
Within *C. flaviventralis*	1	0.72	NA
Within *C. ajijae*	15	0.68	0–1.45
Within *C. rajgadensis* sp. nov.	1	0.24	NA
C. flaviventralis – *C. ajijae*	12	7.33	7.0–7.73
C. flaviventralis – *C. rajgadensis* sp. nov.	4	7.25	7.0–7.49
C. ajijae – *C. rajgadensis* sp. nov.	12	2.29	1.93–2.66

Morphometric analysis

Principal component analysis (PCA) on the 15 variables indicates that the first three variables explained 85.9% of the variation (Table 3). PC1 explained ca. 66% of the variation and described larger body sizes with large and broad heads, and longer legs. PC2 explained ca. 11% of the variation and described broader tails and shorter internasal distance. PC3 explained ca. 9.5% of the variation and was described by shorter and slender trunks. Plotting the first two PCs show that there is considerable difference in the morphospace of the new species compared to *Cnemaspis ajijae* and *C. flaviventralis* along PC1 but not PC2. However, there was partial yet considerable overlap in the morphospace of *C. ajijae* and *C. flaviventralis* along PC1 and PC2. The morphospace of *C. ajijae* and *C. flaviventralis* also showed partial overlap along PC1 and PC3, however, the new species was outside the 95% confidence interval estimated for the morphospace of *C. ajijae* and *C. flaviventralis*. Given these data and those from the phylogenetic analyses we describe the new population from Rajgad fort as a new species and describe the same below.

Table 3. Loadings obtained from the Principal Component Analysis on 15 morphometric variables collected for the *Cnemaspis flaviventralis* clade. Bold values indicate strong loading with correlation > 0.5.

Character	PC1	PC2	PC3
SVL	**0.8825**	-0.0015	-0.3526
TRL	**0.8656**	-0.0582	-0.3186
TW	**0.6393**	-0.4388	**-0.5745**
TLW	-0.0772	**0.6070**	**-0.7035**
HL	**0.8347**	-0.2506	0.0132
HW	**0.8659**	0.3021	-0.1239
HD	**0.8458**	-0.0941	-0.0452
FL	**0.8244**	0.0044	0.1845
TBL	**0.9352**	-0.1007	0.1505
ED	**0.8005**	0.4294	0.2876
EN	**0.9308**	0.1877	-0.0319
ES	**0.9418**	0.2659	0.0089
ET	**0.8876**	0.0467	0.3868
IN	0.4838	**-0.7485**	-0.0733
IO	**0.8894**	0.0548	0.2527
Eigen values	9.8661	1.5932	1.4278
Standard deviation	3.1410	1.2622	1.1949
Proportion of Variance	0.6577	0.1062	0.0952
Cumulative Proportion	0.6577	0.7640	0.8591

Taxonomy

Family Gekkonidae Gray, 1825
Genus *Cnemaspis* Strauch, 1887

Cnemaspis rajgadensis sp. nov.

http://zoobank.org/E4F61F55-8716-4BF3-9951-7728B24056EF

Figs 1–7, Tables 1–5

Type material. ***Holotype*:** India • ♂, adult; Rajgad fort, Pune District, Maharashtra State; 18°14'46N, 73°40'55E; 1324 m a.s.l.; 27 Sept. 2020; Amit Sayyed leg.; BNHS 3100.

***Paratypes*:** India • 2 ♀, adults; same data as for holotype; Abhijit Nale, Kiran Ahire and Mahesh Bandgar leg.; BNHS 3101, 3102.

Diagnosis. A small-sized *Cnemaspis* with adult SVL < 27 mm,; 7–7 supralabials; 6–7 infralabials; dorsal scales heterogeneous with small, granular, weakly keeled scales, intermixed with randomly arranged, weakly keeled, slightly larger tubercles; conical and spine-like tubercles absent on either side of the flanks but a row of enlarged tubercles present on the lower flanks; dorsal paravertebral scales 80–92; mid-dorsal scales 62–67; ventral surface of neck, pectoral, abdominal region, un-

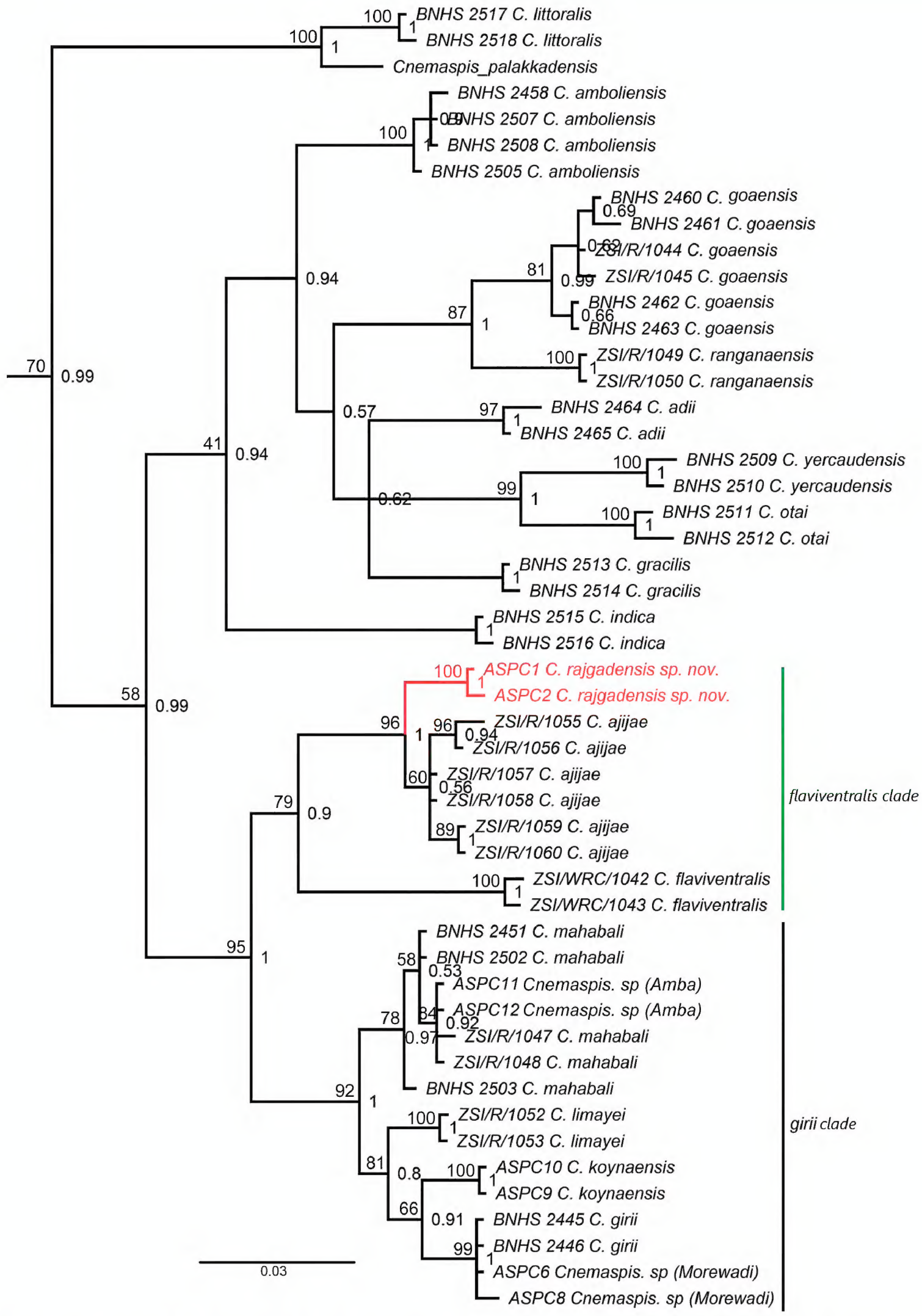

Figure 1. Phylogentic relationships showing the topology from the Bayesian analyses for Indian species of *Cnemaspis* based on 16S rRNA gene. Values along the nodes are bootstrap support values from the Maximum Likelihood analysis and posterior probabilities from the Bayesian analyses respectively. The lineage in red indicates the phylogenetic cluster of the new species.

der limbs, and tail weakly keeled; mid-ventral scales 123–141; ventral scales across mid-body 28–29; subdigital lamellae under fourth digit of manus 15, under fourth digit of pes 18; males with 3 femoral pores and absence of precloacal pores; dorsal scales of tail granular, weakly keeled, roughly same in size and shape to those on mid-body dorsum, gradually becoming larger, flattened, blunt, sub-imbricate posteriorly, intermixed with whorls of slightly enlarged, strongly keeled tubercles; scales on ventral aspect of original tail imbricate, weakly keeled, without a series of enlarged sub-caudal scales, roughly same in size of those on dorsal tail; a single enlarged postcloacal spur on each side.

Comparisons. *Cnemaspis rajgadensis* sp. nov. differs from all other Indian species of *Cnemaspis* by having the following suite of characters: dorsal scales heterogeneous (vs. homogeneous in *C. adii* Srinivasulu et al., 2015, *C. assamensis* Das & Sengupta, 2000, *C. boiei*

Gray, 1842, *C. indica* Gray, 1846, *C. jerdonii* Theobald, 1868, *C. kolhapurensis* Giri et al., 2009, *C. littoralis* Jerdon, 1853, *C. mysoriensis* Jerdon, 1853, *C. nilagirica* Manamendra et al., 2007, *C. palakkadensis* Sayyed et al., 2020, *C. zacharyi* Cyriac et al., 2020); conical and spine-like tubercles absent on flanks (vs. conical or spine-like tubercles on the flanks in *C. amboliensis* Sayyed et al., 2018, *C. anandani* Murthy et al., 2019, *C. assamensis*, *C. flaviventralis* Sayyed et al., 2016, *C. goaensis* Sharma, 1976, *C. gracilis*, *C. jerdoni*, *C. koynaensis* Khandekar et al., 2019, *C. littoralis*, *C. monticola* Manamendra et al., 2007, *C. mysoriensis*, *C. nilagirica*, *C. otai*, *C. rishivalleyensis* Agarwal et al., 2020, *C. stellapulvis* Khandekar et al., 2020); presence of only femoral pores (vs. presence of femoral and precloacal pores in *C. adii*, *C. agarwali* Khandekar, 2019, *C. amboliensis*, *C. andersonii* Annandale, 1905, *C. australis* Manamendra et al., 2007, *C. bangara* Agarwal et al., 2020, *C. goaensis*, *C. gracilis* Beddome, 1870, *C. graniticola* Agarwal et al., 2020, *C. monticola*, *C. otai* Das & Bauer, 2000, *C. rishivalleyensis*, *C. shevaroyensis* Khandekar et al., 2019, *C. thackerayi* Khandekar et al., 2019, *C. wicksii* Stoliczka, 1873, *C. yelagiriensis* Agarwal et al., 2020, *C. yercaudensis* Das & Bauer, 2000; presence of only precloacal pores in *C. aaronbaueri* Sayyed et al., 2019, *C. avasabinae* Agarwal et al., 2020, *C. anamudiensis* Cyriac et al., 2018, *C. beddomei* Theobald, 1876, *C. maculicollis* Cyriac et al., 2018, *C. nairi* Inger et al., 1984, *C. ornata* Beddome, 1870; no femoral or precloacal pores in *C. assamensis* and *C. boiei*; a continuous series of precloacal femoral pores in *C. kolhapurensis*); SVL less than 27 mm (vs. ≥ 40 in *C. aaronbaueri*, *C. anamudiensis*, *C. anandani*, *C. bangara*, *C. beddomei*, *C. chengodumalaensis* Cyriac et al., 2020, *C. graniticola*, *C. heteropholis* Bauer, 2002, *C. kolhapurensis*, *C. kottiyoorensis* Cyriac & Umesh, 2014, *C. maculicollis*, *C. magnifica* Khandekar et al., 2020, *C. nairi*, *C. nilagirica*, *C. ornata*, *C. sisparensis* Theobald, 1876, *C. thackerayi*, *C. wynadensis* Beddome, 1870, *C. yelagiriensis*, *C. zacharyi*).

C. rajgadensis sp. nov. closely resembles members of the *girii* and *flaviventralis* clade, all of which are small-sized species (SVL < 40 mm), have heterogeneous mid-dorsal scales, 2–5 femoral pores in males and having median subcaudals not enlarged. However, the new species can be differentiated from members of the *girii* clade by its higher number of ventral scales (28–29) across mid-body (vs. 22–24 in *C. amba*, 26–28 in *C. girii*, 20–26 in *C. koynaensis*, 26–27 in *C. limayei* and 26–27 in *C. mahabali*), absence of spine-like tubercles on flanks (vs. presence of spine-like tubercles in *C. koynaensis*), presence of enlarged tubercles row on lower flanks (vs. absence of tubercles on lower flanks of *C. girii*, *C. limayei*, *C. mahabali*), and by the weakly keeled ventral scales (vs. smooth in all members of the *girii* clade except *C. mahabali*).

The new species can be differentiated from *C. ajijae* and *C. flaviventralis* by its adult size (< 27 mm SVL vs. > 30 mm in *C. ajijae* and *C. flaviventralis*); short and wider than long head [HL 16.6% of SVL, HW 110.6% of HL] (vs. elongated in *C. flaviventralis* [HL22% of SVL, HW 81.8% of HL]); small eyes [ED 11.4% of HL] (vs. larger eyes in *C. flaviventralis* [ED 18.9% of HL] and *C. ajijae* [ED 21.6% of HL]); short snout [ES 68.7% of HL] (vs. long snout in *C. ajijae* [ES 82.4% of HL]); short and broad trunk [TW 61.5% of TRL](vs. slender in *C. ajijae* [TW 50.8% of TRL]). *C. rajgadensis* sp. nov. can also be differentiated by the weakly keeled ventral scales as opposed to the smooth scales in *C. ajijae* and *C. flaviventralis*.

Description of the holotype. Adult male generally in good state of preservation, albeit slightly dehydrated (Fig. 1A, B). SVL 25.7 mm, head short (HL/SVL 0.16), wide (HW/HL 1.1), not depressed (HD/HL 0.67), distinctly larger from neck. Loreal region slightly inflated, canthus rostralis not prominent. Snout slightly longer than half of head length (ES/HL 0.67); scales on snout and canthus rostralis small, round, weakly keeled, juxtaposed, slightly larger than those on forehead and interorbital region; occipital and temporal region with much smaller, weakly keeled, granular scales (Fig. 2A). Eye small (ED/HL 0.17), with round pupil; supraciliaries not elongate. Tympanum deep, ear-opening very small (EOD/HL 0.04); eye to ear distance much longer then diameter of eye (ET/EOD 11.5). Rostral much wider (1.1 mm) than high (0.4 mm), incompletely divided dorsally by a strongly developed rostral groove; single enlarged supranasal on each side, twice in the size of postnasals, separated from each other by triangular enlarged single internasal scales; rostral in contact with supralabial I, nasal, supranasal and internasal; nostrils small, oval, bordered by postnasals, supranasal and rostral; two rows of small scales separate the orbit from the supralabials (Fig. 2C). Mental enlarged, subtriangular, not pointed posteriorly, wider (1.5 mm) than long (0.9 mm); two pairs of postmentals, inner pair slightly large, separated by single large scale, postmentals bordered posteriorly by eight smaller, rounded scales; gular scales granular, slightly raised; throat scales keeled to weakly keeled, flat(Fig. 2B). Supralabials up to angle of jaw seven on the right and left side; supralabial I largest, slightly decreasing in size posteriorly; infralabials up to angle of jaw seven on the right and left side; infralabial I and II equal in size. Canthal region with 13 scales on both sides; supraciliaries separated by 27 scales at midorbit. Body relatively short, trunk less than half of SVL (TRL/SVL 0.38) without ventrolateral folds. Dorsal scales small, granular, weakly keeled, intermixed with randomly arranged, weakly keeled, slightly larger tubercles which increase in size towards the lower flanks and are more pronounced towards the posterior end (Fig. 1C); dorsal paravertebral scales 80; number of mid-dorsal scales 62; conical and spine-like tubercles absent on either side of the flanks, (Fig. 1E). Granular scales on nape weakly keeled, slightly smaller than those on paravertebral rows, smaller still on occiput. Scales on ventral surface of neck, chest, arm, pes and tail weakly keeled; mid-ven-

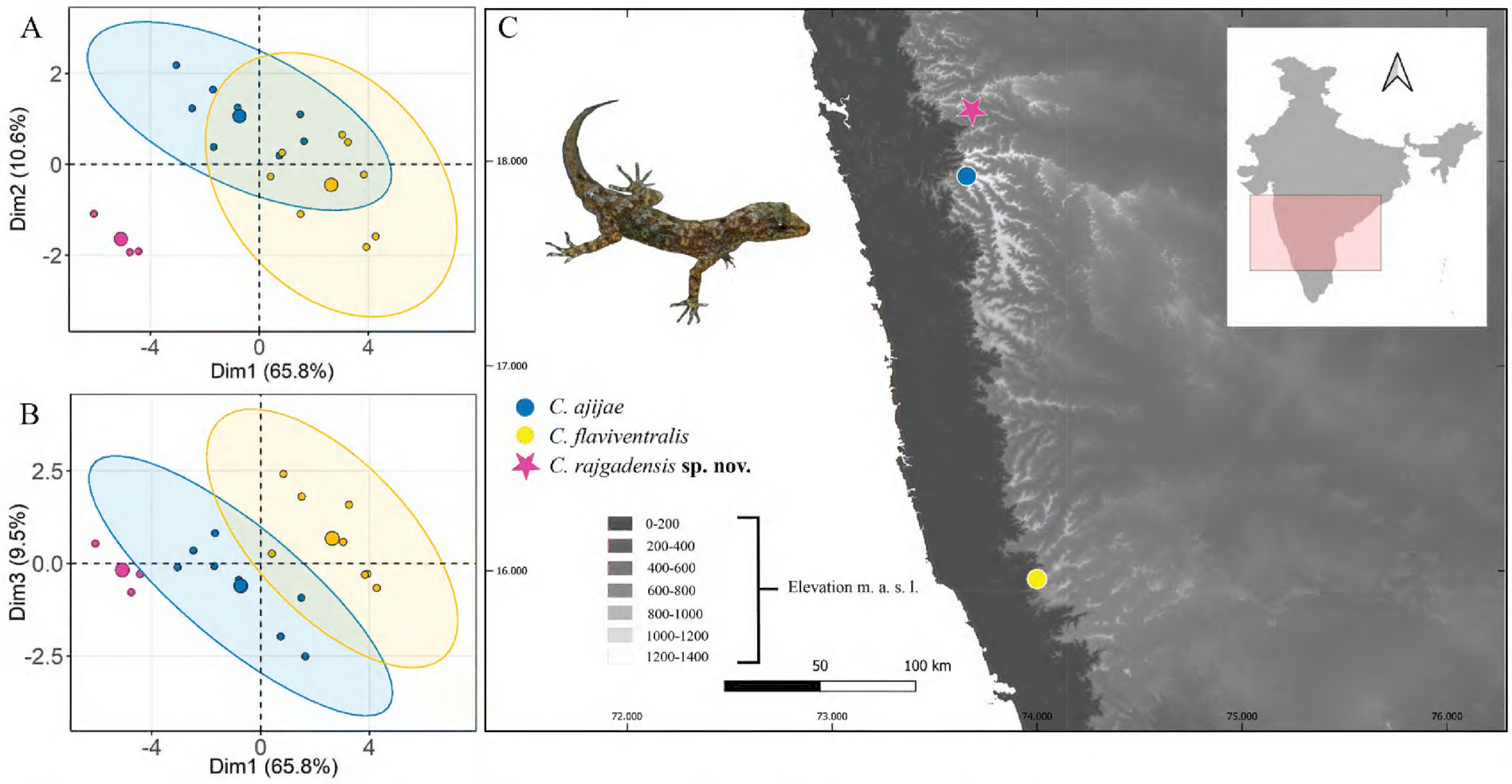

Figure 2. Morphospace occupied by members of the *flaviventralis* clade including *Cnemaspis rajgadensis* sp. nov. as obtained from the Principal Component Analysis (PCA) along with distribution of the species. **A** Plot of PC1 and PC2, **B** Plot of PC1 and PC3, C – Map showing the type localities of *C. rajgadensis* sp. nov., *C. ajijae* and *C. flaviventralis* from northern Western Ghats of India.

tral scales 123, mid-body scales 29 across the ventral between the lowest rows of dorsal scales (Fig. 1D); three femoral pores on each thigh (Fig. 2D),separated by 25 poreless scales; precloacal pores absent; forelimbs moderately long, slender; dorsal scales of brachium weakly keeled, slightly raised; scales of forearm weakly keeled, imbricate, smaller than those on brachials; ventral scales of brachium smooth, rounded, juxtaposed, smaller than those on forearm; scales beneath forearm, weakly keeled, flat, juxtaposed, slightly raised; palmar scales smooth, juxtaposed, raised; claws slightly recurved; dorsal scales of thigh and tibia weakly keeled, imbricate, slightly raised; ventral scales of thigh and tibia flat, imbricate; subtibial scales weakly keeled, imbricate; plantar scales smooth, juxtaposed, raised; digits long with an inflected joint; subdigital lamellae unnotched; lamellae beneath first phalanges slightly widened; slight interdigital webbing (Fig. 2E, F); subdigital lamellae on finger I: 9, finger II: 12, finger III: 15, finger IV: 15, finger V: 12; toe I: 8, toe II: 13, toe III: 18, toe IV: 18 and toe V: 18. Relative length of digits, fingers: IV (2.4 mm) > III (2.2 mm) > V (2.1 mm) > II (1.9 mm) > I (1.6 mm); toes: IV (4.3 mm) > III (3.5 mm) > V (3.3 mm) > II (2.3 mm) > I (1.0 mm). Tail entire and original, cylindrical, moderately slender, flattened beneath, slightly longer than snout-vent length (TL/SVL 1.0) (Fig. 3A, B). Dorsal scales at tail granular, weakly keeled, roughly same in size and shape to those on mid-body dorsum, gradually becoming larger, flattened, blunt, subimbricate posteriorly, intermixed with slightly enlarged, strongly keeled tubercles forming whorls; scales on ventral aspect of original tail imbricate, weakly keeled, without a series of enlarged sub-caudal scales, roughly same in size of those on dorsal tail; scales on tail base slightly smaller, imbricate and smooth, a single enlarged postcloacal spur on each side.

Colouration in life (Fig. 4A, B). Dorsal ground colour of head, body, limbs, and tail light brown to greyish-brown; head with dark-brown marking, supralabials yellow, infralabials brown, supraciliaries brownish-yellow, brille dusty-orange, iris orange coloured, pupil black; dark brown patch between orbit and nostril, dark-brown markings on temporal and occiput; dark-brown triangular mark surrounded by brown dorsally between the forelimb insertion; dorsum with five brown and white diffused vertebral blotches, from forelimb insertion to hind-limb insertion along with yellow scattered dark small blotches on lateral sides of the body; dorsum of forelimb and hind-limb with alternating dark-brown bands with some yellowish blotches; digits with alternating dark-brown and yellow markings; dorsum of original portion of tail with alternating dark-brown and whitish markings; postcloacal spur yellow; ventral surface of body, limbs, and tail off-white, surrounded with mottled brown. Females are dull in overall colouration on dorsal body, ventral surface of body, limbs, and tail white.

Colouration in preservative (Fig. 1A, B). Dorsum of the body, limbs and tail with brown to greyish-brown which turns into dark brown, white and yellow markings in life turns into grey in preservation; ventral side of head, body white, tail greyish-white.

Etymology. The species epithet is derived from the Rajgad fort of Pune District, Maharashtra, India, from where the type series was collected. Rajgad is a hill fort and historical place, which was the capital of the Maratha

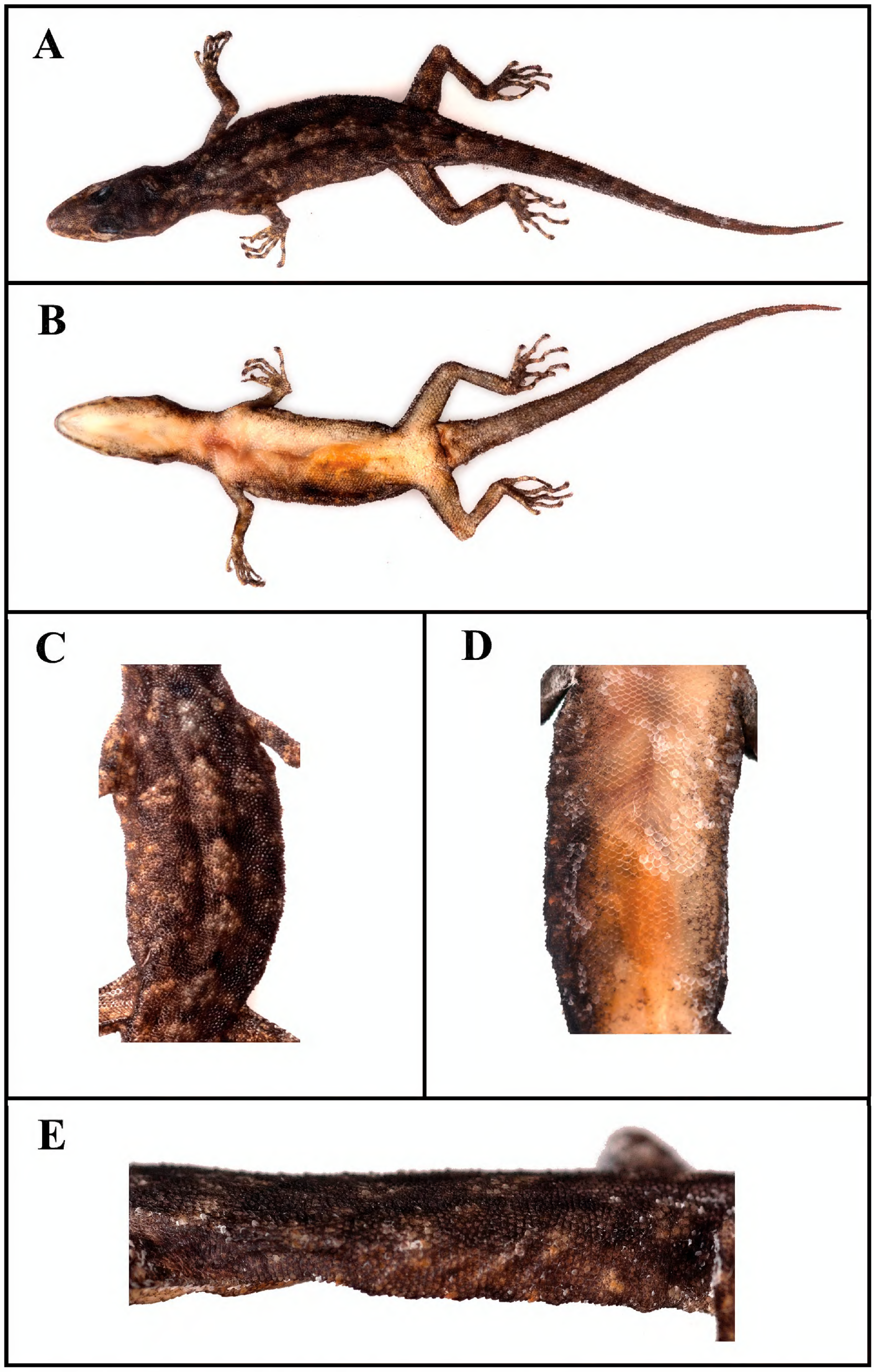

Figure 3. *Cnemaspis rajgadensis* sp. nov. holotype, adult male, SVL 25.7 mm (BNHS 3100): dorsal (**A**), ventral (**B**) views of specimen; dorsal (**C**), ventral (**D**), lateral (**E**) views of mid-body.

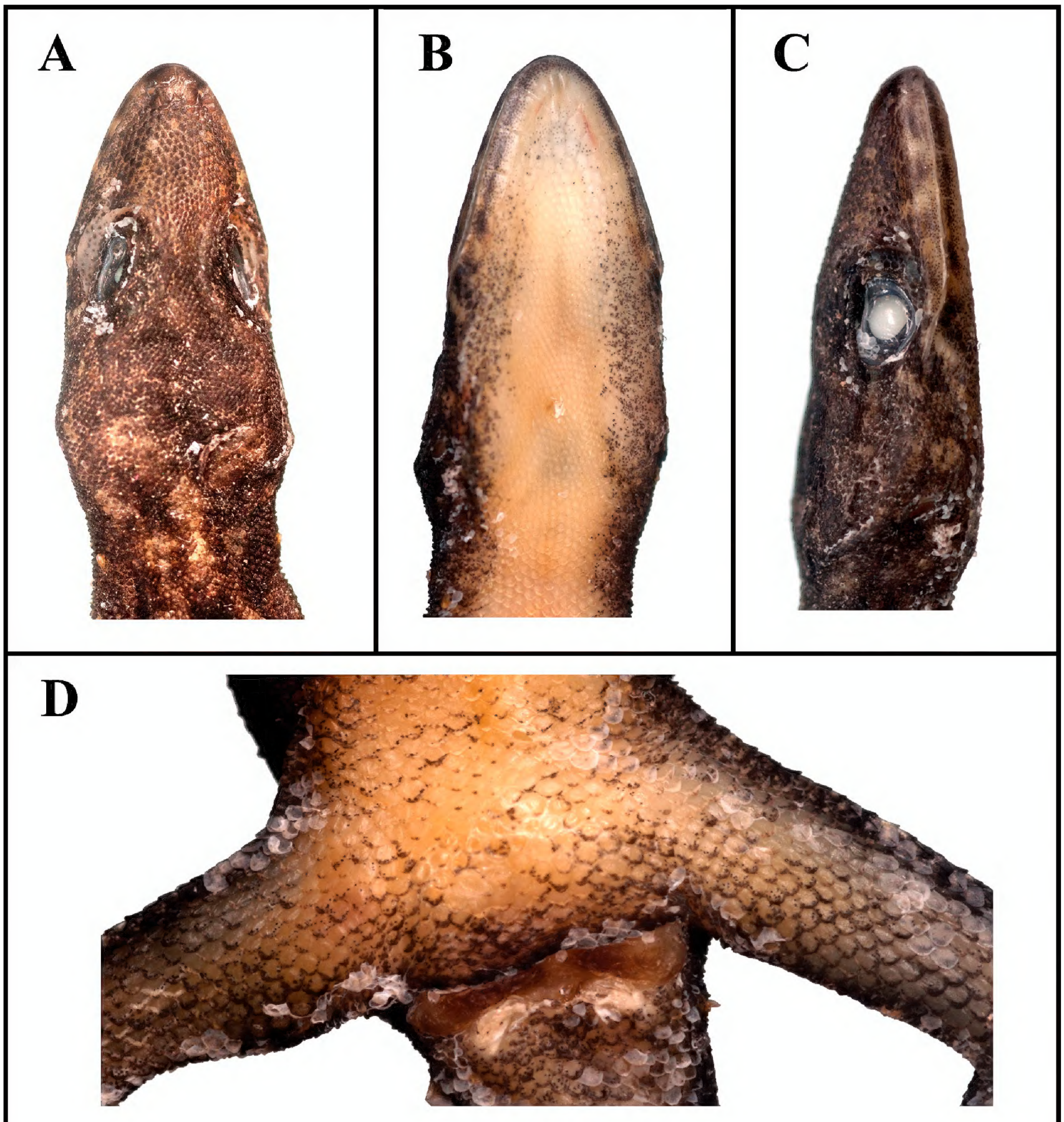

Figure 4. *Cnemaspis rajgadensis* sp. nov., holotype, adult male, SVL 25.7 mm (BNHS 3100): dorsal (**A**), ventral (**B**), lateral (**C**) views of head; view of femoral and precloacal region (**D**); ventral view of lamellae under right menus (**E**) and ventral view of lamellae under right pes (**F**).

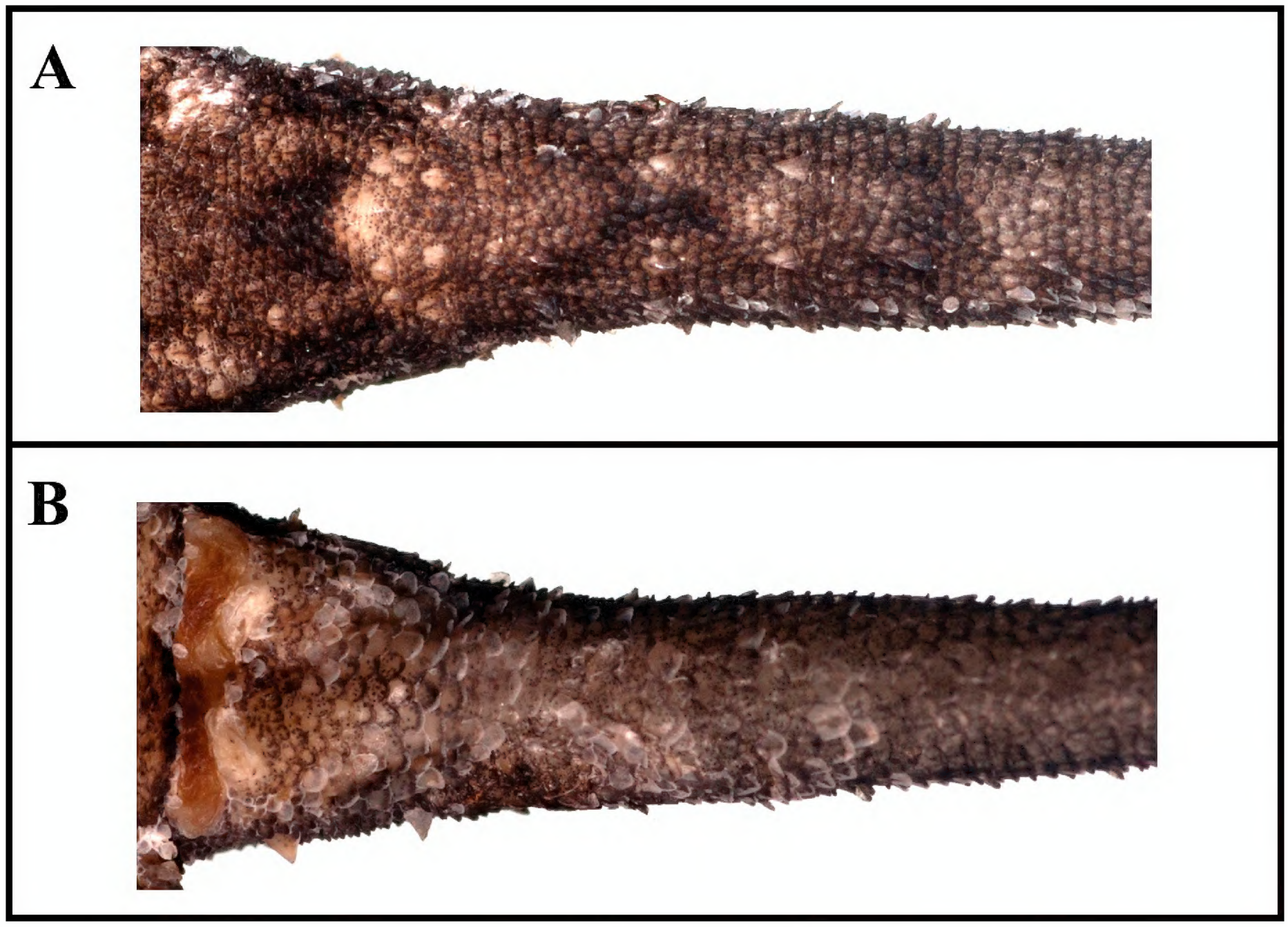

Figure 5. *Cnemaspis rajgadensis* sp. nov. holotype, adult male, SVL 25.7 mm (BNHS 3100): dorsal (**A**) and ventral (**B**) views of tail.

Empire under the rule of Chhatrapati Shivaji Maharaj for almost 26 years during the 17th century. Previously known as Murumdev, Rajgad fort is located around 60 km to the south-west of Pune and about 15 km west of Nasrapur village in the range of northern Western Ghats.

Suggested common name. Rajgad Dwarf Gecko

Distribution. Currently, *Cnemaspis rajgadensis* sp. nov., is known from only its type locality – Rajgad fort, Pune District, Maharashtra.

Natural history. The new species was found on the walls of old stone structure of Rajgad fort (Fig. 5). Rajgad fort is a hill fort situated about 1324 m above the sea level in the Pune district of Maharashtra, India. All the specimens were found active during the evening around 18.30 hrs. at one meter above ground on the rock wall and old man-made structures of the fort, they are uncommon as we encountered only three individuals during our field survey. Both the female specimens collected during our survey were gravid and had two developing eggs each, suggesting that September, when these specimens were collected, falls within the reproductive period of the species. The new species is rupicolous and has currently been observed only on rock structures. Although we were unable to find individuals in the surrounding shrub forest, we presume that the species may also be found in rocky outcrops in the surrounding region. The surrounding area of the fort is covered with grass land slopes and shrub *Strobilanthes callosa* with few large trees. The types were

Table 4. Mensural data for the type series of *Cnemaspis rajgadensis* sp. nov. *= gravid female, ^ = broken tail, – = pores not present. Measurements are in mm.

Measurements	Holotype (BNHS 3100)	Paratype (BNHS 3101)	Paratype (BNHS 3102)
Sex	male	Female*^	Female*
SVL	25.7	26.8	26.3
AG	10.0	12.1	11.5
TW	6.0	7.5	7.2
ED	0.5	0.5	0.5
EN	2.5	2.6	2.6
ES	2.9	3.0	3.1
ET	2.3	2.3	2.3
IN	1.0	1.1	1.1
EOD	0.2	0.2	0.2
HL	4.3	4.3	4.5
HW	4.7	4.8	5.0
HD	2.9	3.3	3.6
IO	2.0	2.3	3.2
UHL	3.0	3.4	3.4
FAL	4.2	3.9	3.5
PAL	4.0	4.0	3.9
PAL	4.0	4.0	3.9
FL1	1.6	1.6	1.4
FL2	1.9	2.0	2.0
FL3	2.2	2.3	2.3
FL4	2.4	3.0	2.9
FL5	2.1	2.2	2.2
FEL	4.6	4.3	4.3
TBL	3.4	4.0	3.9
TOL1	1.0	1.1	1.3
TOL2	2.3	2.0	1.9
TOL3	3.5	4.1	3.6
TOL4	4.3	4.6	3.9
TOL5	3.3	2.7	2.2
TL	27.6	28.9	29.8

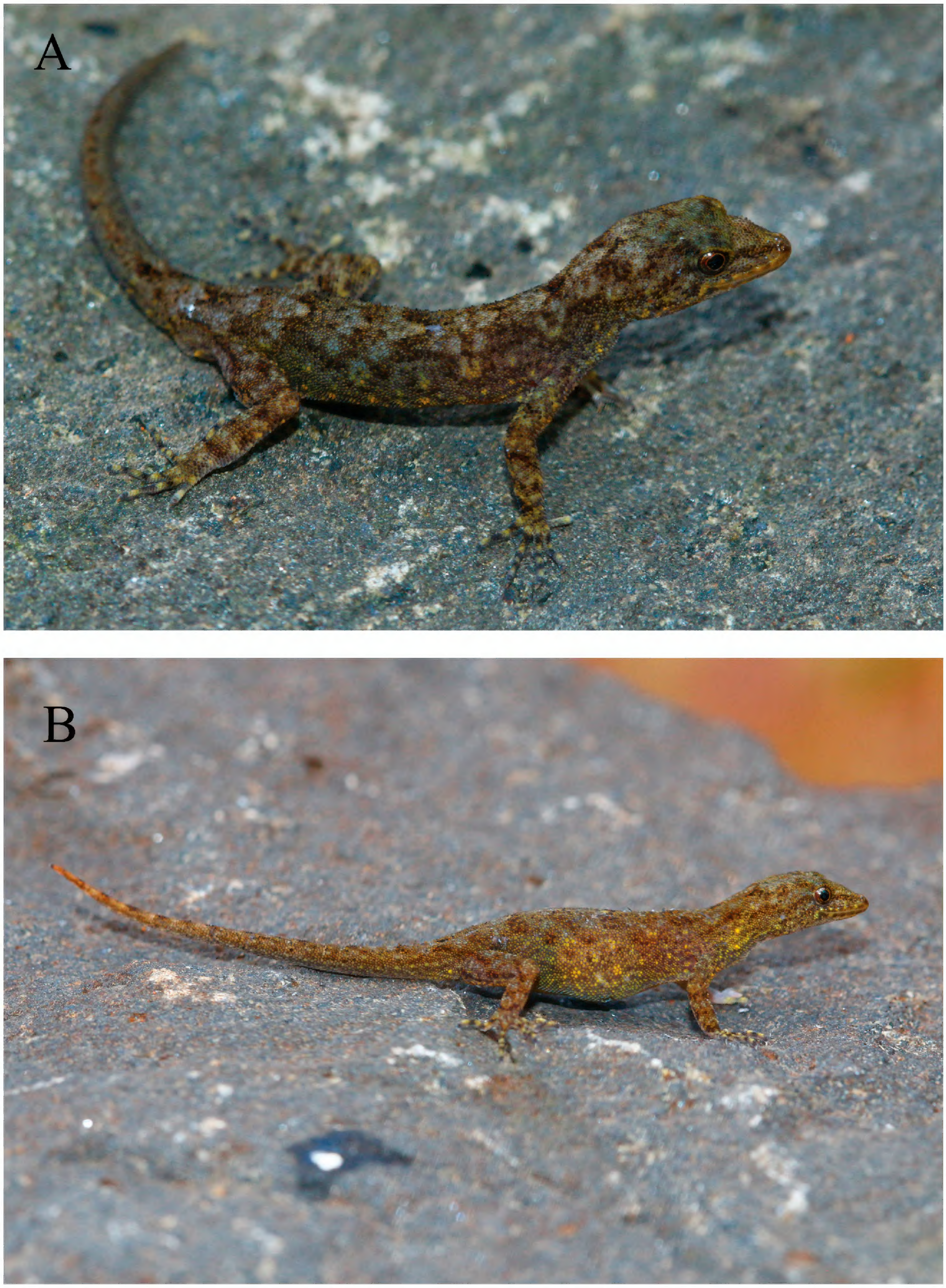

Figure 6. *Cnemaspis rajgadensis* sp. nov. holotype, adult male, SVL 25.7 mm (BNHS 3100) in vivo habitus (**A**) and female in vivo habitus (specimen not collected) (**B**) from type locality.

Figure 7. Habitat of *Cnemaspis rajgadensis* sp. nov. from the type locality at Rajgad fort, Pune, Maharashtra, India.

Table 5. Morphometric and meristic data of the type series of *Cnemaspis rajgadensis* sp. nov. *= gravid female, ^ = broken tail, – = pores not present.

Character	Holotype (BNHS 3100)	Paratype (BNHS 3101)	Paratype (BNHS 3102)
Sex	male	Female*^	Female*
SupL R/L	7/7	7/7	7/7
InfL R/L	7/7	6/6	7/7
SuS	15	16	14
InO	27	29	28
BeT	18	17	17
PoN	2	2	2
PoM	2	2	2
PoP	8	9	9
SuN	3	3	3
CaS	13	13	12
PvS	80	92	86
MbS	62	67	65
MvS	123	127	141
BlS	29	28	29
FPores	3/3	–	–
PS	25	–	–
MLam R	9/12/15/15/12	10/12/15/15/14	9/12/15/15/13
PLam R	8/13/18/18/18	9/13/18/18/18	9/13/16/18/18

found sympatrically with *Cyrtodactylus deccanensis* (Günther), *Hemidactylus maculatus* Dumeril & Bibron and *Hemidactylus* cf. *murrayi* Gleadow.

Discussion

The topology of our phylogenetic tree based on the 16S rRNA gene was more similar to that of Cyriac et al. (2020) than that of Sayyed et al. (2018). We found some discordance in the tree topology between the ML and Bayesian analyses, particularly in the position of the *Cnemaspis amboliensis*. The phylogenetic position of *C. amboliensis* was sister to the *goaensis* + *gracilis* clade with strong support in the Bayesian analysis. However, relationships between *C. amboliensis* + *goaensis* + *gracilis* were unresolved with low support values in the ML analysis. Both ML and Bayesian analyses found *C. rajgadensis* sp. nov. to be sister to *C. ajijae*, which together formed a sister relationship with *C. flaviventralis*. This clade, was deeply divergent from members of the *girii* clade and had higher number of ventral scales across mid-body (28–30) compared to members of the *girii* clade (22–28). Thus, we ascribe the *C. flaviventralis*, *C. ajijae* and *C. rajgadensis* sp. nov. to the *flaviventralis* clade. Interestingly the two new sequences generated in this study (ASPC11, ASPC12) from Amba village were found to be nested within the cluster of *C. mahabali* (BNHS 2451, BNHS 2451) collected from Bhira with moderate to strong support (Fig. 1). Sayyed et al. (2018) reported *C. mahabali* to be a widespread species inhabiting costal hill forests and hilly regions from Tane in the north to Ratnagiri district in the south. The current specimens from Amba, in Kolhapur district, are morphologically similar to *C. mahabali* and shows little to no genetic divergence between specimens of *C. mahabali* (uncorrected p distance = 0–0.4%). Based on these, we confirm the presence of *C. mahabali* in Amba village of Kolhapur district extending the known range of the species.

The new species, *Cnemaspis rajgadensis* sp. nov., would be the twelfth species of the genus *Cnemaspis* described from the northern Western Ghats, India, thus underlining the endemic diversity of this region. This study also marks the importance of forts in Maharashtra as potential habitats for micro fauna. Although these forts are man-made intrusions into natural habitats, these sites being of archaeological importance, are currently protected by law from extensive infrastructure and developmental activities. Most of these forts being situated in the rugged terrains of the northern Western Ghats remain inaccessible to extensive human activities and potentially have conservation implications in protecting local flora and fauna. The current study, as well as the recently described *C. ranganaensis* from Rangana fort, near Kolhapur, Maharashtra indicate rich bio-diversity of endemic reptile fauna occupying these forts. These forts have always been protected for their archaeological importance, however have never been considered for conservation due to lack of data. Dedicated biodiversity surveys of these forts in Maharashtra is much needed to highlight the importance of these structures and also draft appropriate conservation plans.

Acknowledgements

We thank all the reviewers for their constructive comments, which greatly improved the manuscript. We thank the Forest Departments of Maharashtra for issuing collecting permits and for their support during field surveys. We also thank the Institute of Natural History Education and Research (INHER), Pune for institutional facilities and partial funding received for this study. We thank Gaurang Gowande and Shubhankar Deshpande for their help in this study. We are grateful to President, INHER, Pune for the encouragement. We thank Deepak Apte and Rahul Khot, of the Bombay Natural History Society, Mumbai for providing access to specimens and registration of the type specimens. We also thank Vithoba Hegde BNHS, Abhijit Nale, Kiran Ahire, Vikas Jagtap, Mahesh Bandgar, Hrushikesh Awale, Ayaan Sayyed and Masum Sayyed the team Wildlife Protection and Research Society (WLPRS) for their help during fieldwork.

References

Agarwal I, Thackery T, Pal S, Khandekar A (2020) Granite boulders act as deep-time climate refugia: A Miocene divergent clade of rupicolous *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae) from the Mysore Plateau, India, with descriptions of three new species. Journal of Zoological Systematics and Evolutionary Research 00: 1–28. https://doi.org/10.1111/jzs.12391

Akkalkot S (2009) Durga. Marathi language (2nd edn.). Sihyadri Durgabhraman Mandal, India, 644 pp.

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17: 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Cyriac VP, Palot MJ, Deuti K, Umesh PK (2020) A preliminary 16S rRNA phylogeny of the Indian *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae) with the description of two new cryptic species from the *C. wynadensis* clade. Vertebrate Zoology 70(2): 171–193.

Deepak V, Giri VB, Asif M, Dutta SK, Vyas R, Zambre AM, Bhosale HK, Karanth P (2016) Systematics and phylogeny of *Sitana* (Reptilia: Agamidae) of Peninsular India, with the description of one new genus and five new species. Contributions to Zoology 85(1): 67–111. https://doi.org/10.1163/18759866-08501004

Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. https://doi.org/10.1093/nar/gkh340

Giri VB, Bauer AM, Gaikwad KS (2019a) A new ground-dwelling species of *Cnemaspis* Strauch (Squamata: Gekkonidae) from the northern Western Ghats, Maharashtra, India. Zootaxa 2164: 49–60. https://doi.org/10.11646/zootaxa.2164.1.5

Giri VB, Deepak V, Captain A, Pawar S, Tillack F (2019b) A new species of *Boiga* Fitzinger, 1826 (Serpentes: Colubridae) from the northern Western Ghats of India. Journal of Bombay Natural History Society 116: 1–11.

Gunawardene NR, Daniels AED, Gunatilleke IAUN, Guna-tilleke CVS, Karunakaran PV, Nayak KG, Prasad S, Puy-ravaud P, Ramesh BR, Subramanian KA, Vasanthy G (2007) A brief overview of the Western Ghats-Sri Lanka biodiver-sity hotspot. Current Science 93: 1567–1572.

Jog SR, Wakhare A, Chaudhuri S, Unde M, Pardeshi SD (2002) Maharashtra landscape: A perspective, In: Diddee J, Jog SR, Kale VS, Datye VS (Eds) Geography of Maharashtra. Rawat Publications, India, 19–57.

Khandekar A (2019) A new species of rock-dwelling *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae) from Tamil Nadu, southern India. Zootaxa 4571: 383–397. https://doi.org/10.11646/zootaxa.4571.3.6

Khandekar A, Thackery T, Agarwal I (2019) Two more new species of *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae) from the northern Western Ghats, Maharashtra, India. Zootaxa 4656: 043–070. https://doi.org/10.11646/zootaxa.4656.1.2

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29(6): 1695–1701. https://doi.org/10.1093/molbev/mss020

Mirza ZA, Pal S, Bhosale HS, Sanap RV (2014) A new species of gecko of the genus *Cnemaspis* Strauch, 1887 from the Western Ghats, India. Zootaxa 3815: 494–506. https://doi.org/10.11646/zootaxa.3815.4.2

Myers N, Mittermeier RA, Mittermeier CG, Da Fonesca GA, Band Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853–858. https://doi.org/10.1038/35002501

Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Molecular Biology and Evolution 32: 268–274. https://doi.org/10.1093/molbev/msu300

Padhye AD, Sayyed A, Jadhav A, Dahanukar N (2013) *Raorchestes ghatei*, a new species of shrub frog (Anura: Rhacophoridae) from the Western Ghats of Maharashtra, India. Journal of Threatened Taxa 5(15): 4913–4931. https://doi.org/10.11609/JoTT.o3702.4913-31

Pai M (2005) The Western Ghats. Published by Mohan Pai, 237 pp.

Pal S, Gaikwad K, Murthy C, Dutta S, Giri V (2013) New locality records of the recently described gecko *Hemidactylus aaronbaueri* Giri, 2008 with additional notes on natural history. Hamadryad 36: 162–167.

Palumbi SR, Martin A, Romano S, Mcmillan WO, Stice L, Grabowski G (1991) The simple fool's guide to PCR, version 2.0. Honolulu, University of Hawaii.

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67(5): 901–904. [syy032] https://doi.org/10.1093/sysbio/syy032

Ronquist F, Teslenko M, Mark PVD, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Sayyed A, Pyron RA, Dahanukar N (2016) *Cnemaspis flaviventralis*, a new species of gecko (Squamata: Gekkonidae) from the Western Ghats of Maharashtra, India. Journal of Threatened Taxa 8(14): 9619–9629. https://doi.org/10.11609/jott.2599.8.14.9619-9629

Sayyed A, Pyron RA, Dileepkumar R (2018) Four new species of the genus *Cnemaspis* Strauch,(Sauria: Gekkonidae) from the northern Western Ghats, India. Amphibian & Reptile Conservation 12(e157): 1–29.

Sayyed A, Cyriac VP, Dileepkumar R (2020) A new cryptic species of *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae) belonging to the *C. littoralis* complex from Anakkal, Palakkad, Kerala, India. Amphibian & Reptile Conservation 14(3): 31–45. [e251]

Sayyed A, Sulakhe S (2020) A new *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae) from the northern Western Ghats, Maharashtra, India. Zootaxa 4885(1): 83–98. https://doi.org/10.11646/zootaxa.4885.1.5

Sharma RC (1976) Records of the reptiles of Goa. Records the Zoological Survey of India 71(1975): e149167.